

Claims

1. A polypeptide fragment capable of raising a specific T-cell response, said fragment comprising a peptide consisting of at least 9 consecutive amino acid residues of ML-IAP (SEQ ID NO:1), wherein said peptide is selected from the group consisting of rlqeertck (SEQ ID NO:245), qilgqlrpl (SEQ ID NO:55), Itaevppel (SEQ ID NO:100), gmgseelrl (SEQ ID NO:84), elptprrev (SEQ ID NO:200), rlqeertckv (SEQ ID NO:297), qlcpicrapv (SEQ ID NO:298), llrskggrdfv (SEQ ID NO:300), vleppgardv (SEQ ID NO:301) and pitaevppel (SEQ ID NO:302);
and wherein said polypeptide fragment comprises at the most 15 amino acids.
2. The polypeptide fragment according to claim 1 comprising or consisting of at least 10 consecutive amino acid residues of ML-IAP (SEQ ID NO:1).
- 15 3. The polypeptide fragment according to claim 1, wherein said polypeptide fragment comprises at the most 10 amino acids.
4. The polypeptide fragment according to any of claims 1 to 3, wherein the specific T-cell response is measured as more than 50 peptide specific spots per 10^6 cells
20 in an ELISPOT assay performed without pre-stimulation in vitro.
5. The polypeptide fragment according to any of claims 1 to 3, wherein the specific T-cell response is measured as more than 50 peptide specific spots per 10^5 cells in an ELISPOT assay performed after stimulation in vitro.
- 25 6. The polypeptide fragment according to any of claims 1 to 3, wherein the specific T-cell response is measured as more than 50 peptide specific spots per 10^6 cells in an ELISPOT assay performed using PBL from an individual that has not been subjected to immune therapy against a neoplastic disease.
- 30 7. The polypeptide fragment according to any of claims 1 to 3, wherein the polypeptide fragment is characterised by having a C_{50} value, measured as the concentration (μM) of the polypeptide fragment required for half maximal binding to a MHC (Major Histocompatibility Complex) class I molecule.

8. The polypeptide fragment according to claim 7, wherein the polypeptide fragment is characterised by having a C_{50} value, measured as the concentration (μM) of the polypeptide fragment required for half maximal binding to an MHC class I molecule, of less than 1000.
5
9. The polypeptide fragment according to any of claims 7 and 8, wherein the polypeptide fragment is characterised by having a C_{50} value, measured as the concentration (μM) of the polypeptide fragment required for half maximal binding to an MHC class I molecule, of less than 100.
10
10. A polypeptide fragment according to any of claims 7 to 9, wherein the polypeptide fragment is characterised by having a C_{50} value, measured as the concentration (μM) of the polypeptide fragment required for half maximal binding to an MHC class I molecule, of less than 31.
15
11. A polypeptide fragment according to any of claims 7 to 10, wherein the polypeptide fragment is characterised by having a C_{50} value, measured as the concentration (μM) of the polypeptide fragment required for half maximal binding to an MHC class I molecule, of less than 5.
20
12. A polypeptide fragment according to any of claims 1 to 11, wherein the fragment is capable of activating T-cell growth in vitro.
25
13. A polypeptide fragment according to claim 12, wherein the fragment is capable of activating T-cell growth in vitro so that more than 10^5 antigen specific CTLs may be harvested after 4 stimulation cycles starting with 10^4 PBMC
14. A method of selecting a peptide comprising a fragment of ML-IAP for use in a vaccine composition comprising the steps of
30
 - i) Providing an individual who has not been subjected to immune therapy
 - ii) Providing a polypeptide fragment comprising a peptide consisting of at least 9 consecutive amino acid residues of ML-IAP (SEQ ID NO:1),
 - iii) Testing specific T-cell responses against fragments of ML-IAP in said individual
- 35

- iv) Selecting fragments of ML-IAP wherein said T-cell response corresponds to or is better than a predetermined selection criterium.
15. The method according to claim 14, wherein said peptide is selected from the group consisting of rlqeertck (SEQ ID NO:245), qilgqlrl (SEQ ID NO:55), Itaevppel (SEQ ID NO:100), gmgseelrl (SEQ ID NO:84), elptprrev (SEQ ID NO:200), rlqeertckv (SEQ ID NO:297), qlcpicrapv (SEQ ID NO:298), llrskgrdfv (SEQ ID NO:300), vleppgardv (SEQ ID NO:301) and pitaevppel (SEQ ID NO:302).
- 10 16. The method according to claim 15, wherein said polypeptide fragment comprises at the most 15 amino acids.
- 15 17. The method according to claim 14, wherein testing said T-cell response comprises an ELISPOT assay.
18. The method according claim 17, wherein said predetermined selection criterium is more than 50 peptide specific spots per 10^6 cells in said ELISPOT assay.
- 20 19. A polypeptide fragment according to any of claims 1 to 13 for use as a medicament.
- 25 20. Use of one or more polypeptide fragments according to any of claims 1 to 13 in the manufacture of a medicament for treatment of a clinical condition in an individual in need thereof.
21. Use according to claim 20, wherein said clinical condition is cancer.
22. Use according to claim 21, wherein the cancer is malignant melanoma.
- 30 23. Use according to claim 20, wherein said clinical condition is an auto-immune disease.
- 35 24. Use according to any of claims 20 to 23, wherein at least one of said polypeptide fragments is restricted to an HLA molecule present in said individual.

25. Use according to any of claims 20 to 24, wherein said individual has not previously been subjected to immune therapy against a neoplastic disease.
- 5 26. A medicament for treating a clinical condition comprising a polypeptide according to any of claims 1 to 13 as an active ingredient.
- 10 27. A vaccine composition comprising isolated ML-IAP (SEQ ID NO:1) and/or one or more fragments thereof and a pharmaceutically acceptable carrier and/or adjuvant.
28. The vaccine composition according to claim 27, wherein said composition comprises at least one polypeptide fragment according to any of claims 1 to 13.
- 15 29. The vaccine composition according to any of claims 27 to 28 further comprising an adjuvant.
30. The vaccine composition according to claim 29, wherein the adjuvant is selected from the group consisting of Montanide ISA-51 and QS-21
- 20 31. The vaccine composition according to any of claims 27 and 28, wherein the composition further comprises a carrier.
- 25 32. The vaccine composition according to claim 31, wherein the carrier is a dendritic cell.
- 30 33. The vaccine compositions according to claim 27 to 28, wherein the composition comprises more than one different ML-IAP fragment according to any of claims 1 to 13.
34. The vaccine composition according to claim 33, wherein the composition comprises different ML-IAP fragments, wherein said fragments are capable of associating with the most frequently occurring MHC class I molecules.

35. The vaccine composition according to claim 33, wherein the composition comprises at least 2 different ML-IAP fragments each capable of associating with a different HLA molecule selected from the group consisting of HLA-A2, HLA-A1, HLA-A3, HLA-A24, HLA-B7, HLA-B27 and HLA-B44.

5

36. The vaccine composition according to claim 35, wherein the composition comprises at least one class I-restricted ML-IAP peptide and at least one class II-restricted ML-IAP peptide.

10

37. A pharmaceutical composition comprising the vaccine composition according to any of claims 27 to 36 and an anti-cancer medicament.

38. The pharmaceutical composition according to claim 37, wherein the anti-cancer medicament is selected from the group consisting of chemotherapeutic agents and immunotherapeutic agents.

15

39. A kit-of-parts comprising comprising ML-IAP (SEQ ID NO:1) and/or one or more fragments thereof of and a bioactive compound selected from the group consisting of a chemotherapeutic agent, an immunotherapeutic agent, and a second cancer vaccine composition.

20

40. The kit-of-parts according to claim 39 comprising one or more polypeptide fragments according to any of claims 1 to 13.

25

41. A method for treatment of an individual diagnosed with cancer, said method comprising the step of administering to the individual the polypeptide fragment according to any of claims 1 to 13, or the vaccine composition according to any of claims 27 to 36, or the pharmaceutical composition according to any of claims 37 and 38 or the kit of parts according to any of claims 39 and 40.

30

42. The method according to claim 41, wherein said individual has not previously been subjected to immune therapy against a neoplastic disease.

43. The method according to claim 41, wherein said cancer is malignant melanoma.

35

44. A method for prophylactic treatment of an individual at risk of developing a cancer, said method comprising the step of administering to the individual the polypeptide fragment according to any of claims 1 to 13, or the vaccine composition according to any of claims 27 to 36, or the pharmaceutical composition according to any of claims 37 and 38 or the kit of parts according to any of claims 39 and 40.
- 5
45. A method for raising a specific T-cell response against an epitope of ML-IAP (SEQ ID NO:1) in an individual, said method comprising the steps of administering to the individual a polypeptide fragment according to any of claims 1 to 13, and raising a specific T-cell response against an epitope of ML-IAP in the individual.
- 10
46. The method according to claim 45, wherein the methods comprises administering one or more polypeptide fragments according to any of claims 1 to 13, and wherein at least one fragment is restricted to an HLA molecule present in said individual.
- 15
47. An antibody capable of specific recognition of a polypeptide fragment according to any of claims 1 to 13.
- 20
48. A method for activating and expanding T-cells specific for ML-IAP or fragments thereof comprising the steps of co-cultivating T-cells and ML-IAP or one or more fragments thereof.
- 25
49. The method according to claim 48, wherein "ML-IAP or fragments thereof" is one or more polypeptide fragments according to any of claims 1 to 28.
50. The methods according to any of claims 48 and 49, wherein the method comprises generating and loading monocyte-derived dendritic cells (DC) with ML-IAP fragment(s) and co-cultivating said DC and perifiral blood monocytes (PBMC) comprising T-cells.
- 30
51. The methods according to any of claims 48 and 49, wherein the method comprises generating *Drosophila melanogaster* cells expressing one or more different
- 35

85

HLA molecules, loading said *Drosophila melanogaster* cells with ML-IAP fragment(s) and co-cultivating said *Drosophila* cells with perifiral blood monocytes (PBMC) comprising T-cells or T-cells purified from PBMC.

5 52. ML-IAP specific T-cells obtained by the method according to any of claims 48 to
51.

53. T-cells according to claim 52, wherein said ML-IAP specific T-cells are cytotoxic
T-cells.

10 54. Use of ML-IAP specific T-cells according to any of claims 52 and 53 for the
preparation of a medicament for treatment of a clinical condition in an individual
in need thereof.

15